that deform the underlying membrane by progressive recruitment of clathrin, adaptors and other regulatory proteins. They ultimately close off and bud inward to form coated vesicles. Coated plaques are larger, less sharply curved, longer-lived structures; their clathrin lattices do not close off, but instead move uniformly inward from the cell surface shortly before membrane fission. Local remodeling of actin filaments is essential for the formation, inward movement and dissolution of plaques, but it is not required for normal formation and budding of coated pits. We conclude that there are at least two distinct modes of clathrin coat formation at the plasma membrane – classical coated pits and coated plaques – and that these two assemblies interact quite differently with other intracellular structures.

**2932-Plat**
**The Language of Shape: Biological Reactions are Dramatically Affected by the Shape of Lipid Membranes**
**Dimitrios Stamou**.
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A plethora of biological process are taking place on the surface of lipid membranes. As a rule membranes in vivo are curved in a variety of complex geometries. Here I will present a quantitave study on the influence of membrane curvature on protein-membrane and membrane-membrane interactions. To gain systematic access to a continuum of membrane curvatures we immobilized liposomes on a surface at dilute densities. Using confocal fluorescence microscopy we imaged single liposomes of different size, and therefore different curvature, and monitored their interaction with a binding partner (proteins or other liposomes).
I will discuss unpublished data on two important classes of biomolecular interactions that exhibited dramatic curvature dependence: A) SNARE-mediated docking and fusion B) anchoring of peripheral proteins.
The following references provide partial information on the single-liposome assay:
B. Lohse et al., JACS. in press.
A. H. Kunding et al., Biophysical Journal. 2008. 95 (3).
S. M. Christensen and D. Stamou. Invited review Soft Matter, Cover Page Article. 2007. 3 (7)
D. Stamou et al. Angewandte Chem.-Int. Edition, Cover Page Article. 2003. 42 (45).

**2933-Plat**
**Cortical Tension Affects the Spatial Heterogeneity of Clathrin-Coated Pit Dynamics**
**Allen P. Liu**, Dinah Loerke, Sandra L. Schmid, Gaudenz Danuser.
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Clathrin-mediated endocytosis (CME) in mammalian cells is critical for many cellular processes including cell surface receptor down-regulation and nutrient uptake. From analyses of protein interaction networks, the actin polymerization machinery is a modular component within the endocytic interactome. However, the precise role of actin in CME is still under debate. Live cell microscopy has revealed a wide variation in the dynamics of clathrin-coated pits (CCPs). To gain insight of the heterogeneity of CCP dynamics and how cortical actin might influence this heterogeneity, we applied total internal reflection fluorescence microscopy to live cells grown on micro-fabricated substrates patterned with adhesive and non-adhesive regions. Cells on patterns showed overall longer CCP lifetimes compared to cells on chemically uniform surfaces, possibly the result of increased cortical tension. CCP lifetime distributions were also significantly different between adhesive and non-adhesive regions. When the structure of cortical actin is weakened by application of an actin monomer sequestering drug latrunculin A (latA), we found that the CCP lifetimes were homogenized to the level of the non-adherent regions. The decrease in CCP lifetime on adherent regions suggests that cortical actin filaments act as barriers at the adherent surface in CME.

**2934-Plat**
**Screening the Sensing of Membrane Curvature by BAR domains on Single Liposome Arrays**
**Vikram K. Bhatia**, Kenneth L. Madsen, Pierre-Yves Bolinger, Per Hedegård, Ulrik Gether, Dimitrios Stamou.
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Membrane traffic relies on the preferential binding of protein domains to high curvature areas. The BAR domain is a banana shaped α-helical homodimer found in several proteins families that play a major role in endocytosis, actin regulation and signaling.[1] It is shown to sense and/or induce lipid membrane curvature by peripheral binding. While most attention have been aimed at curvature induction[2], we investigate the molecular mechanism of curvature *sensing* by performing a thorough study on the whole superfamily of BAR domain proteins including NBARs, FBARs, IBARs. We compared the sensing properties of 9 different BAR proteins and also measured on numerous truncation or point mutation variants.
We developed a high-throughput single liposome assay[3] to test the curvature dependent binding properties of these BAR proteins. Fluorescence intensities of immobilized vesicles allowed us to measure accurately their size/curvature and the respective densities of BAR proteins. Combining selectivity curves with the mutagenesis studies enabled us to evaluate the contribution of dimer structure, electrostatics and helix insertion to membrane curvature sensing by BAR domain proteins.
Our results prompt a thorough reevaluation of the membrane curvature *sensing* mechanism of BAR domain proteins.
[1] McMahon, H. T. & Gallop, J. L. Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 438, 590-596 (2005).
[2] Frost A. *et al.* Structural Basis of Membrane Invagination by F-BAR domains, Cell, 132, 807-817 (2008).
[3] Stamou, D., Duschl, C., Delamarche, E. & Vogel, H. Self-assembled microarrays of attoliter molecular vessels. Angewandte Chemie-International Edition, Cover Page Article 42, 5580-5583 (2003).

**2935-Plat**
**Computational Delineation of the Bioenergetics of Protein-Mediated Orchestration of Membrane Vesiculation in Clathrin-Dependent Endocytosis**
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Internalization of extracellular cargo by eukaryotic cells via the clathrin-dependent endocytosis (CDE) is an important regulatory process prominent in several cellular functions. Subsequent to receptor activation, a sequence of molecular events in CDE is responsible for the recruitment of various accessory proteins such as AP-2, epsin, AP180, eps15, dynamin, amphiphysin, endophilin, and clathrin to the plasma membrane to orchestrate membrane vesiculation. While the involvement of these proteins have been established and their roles in membrane deformation, cargo recognition, and vesicle scission have been identified, current conceptual understanding falls short of a mechanistic description of the cooperativity and the bioenergetics of the underlying vesicle nucleation event which we address here using theoretical models based on an elastic continuum representation for the membrane and atomistic to coarse-grained representations for the proteins. We employ the surface evolution approach to describe membrane geometries by minimizing the Helfrich Hamiltonian in a curvilinear coordinate system and address how the energetics of vesicle formation in a membrane is impacted by the presence of a growing clathrin coat. We consider two limiting scenarios: (1) the clathrin assembly model in which the clathrin coat induces membrane curvature by forming a curvilinear scaffold; (2) the accessory curvature-inducing protein assembly model, in which the clathrin lattice merely serves as a template to spatially pattern curvature inducing proteins such as epsin which collectively induce membrane curvature. Analyzing the energy required for vesicle formation from a planar bilayer, we demonstrate the role of the CDE protein assembly in driving membrane vesiculation. Furthermore, using a time-dependent Ginzburg-Landau formalism along with the thermodynamic method of free energy perturbation, we calculate the free energy the nucleated vesicle and quantify the finite-temperature corrections to the energy landscape of vesicle nucleation in CDE.

**2936-Plat**
**The Dynamics Of Secretion-associated Plasma Membrane Changes Visualized With Polarized TIRFM**
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The morphological dynamics of the plasma membrane were visualized in bovine adrenal chromaffin cells using polarized total internal reflection fluorescence microscopy (TIRFM). This method is based on monitoring the fluorescence of an oriented membrane probe (the carbocyanine dye, DiI) excited by a polarized evanescent field created by TIR illumination. DiI has been shown to embed in the membrane with its transition dipole moments nearly in the plane of the membrane. Thus, by monitoring the pixel-by-pixel ratio of the membrane-embedded DiI fluorescence excited by the two polarizations (***p*** - perpendicular to substrate; ***s*** - parallel to substrate) over time, regions of membrane curvature are vividly highlighted. To relate the orientation of the membrane with exocytosis, granules were labeled with the marker neuropeptide (NPY) - cerulean. In response to high KCl depolarization, fusion of granules coincided with 15-20% increases in DiI-membrane *p/s* values at locations of NPY-Cer release. The *p/s* values then often declined over several seconds to approximately pre-fusion levels. In other instances, the *p/s* values declined more slowly providing evidence of longer-lasting membrane curvature. Some granules were associated with areas of the membrane with increased curvature (larger *p/s* values) prior to stimulation. These granules were significantly more

likely to undergo exocytosis than those without such associations. When endocytosis was inhibited with Dynasore (inhibitor of dynamin 1 and 2), membrane *p/s* changes following fusion were long-lived (10s of seconds). This is consistent with endocytosis occuring at the site of exocytosis. These experiments are the first to directly visualize plasma membrane changes occuring with secretion. They reveal an association of the granule and plasma membrane for seconds before exocytosis and provide direct evidence for maintained curvature of the plasma membrane after exocytosis.

**2937-Plat**

**Actin Cytoskeleton Controls Movement Of Intracellular Organelles In Epithelia**

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**Background:** Intracellular organelles move along microtubules and filamentous actin (F-actin) using molecular motors. Previously we reported that movement of secretory granules was hindered by $Ca^{2+}$-dependent formation of a dense cytoplasmic F-actin meshwork in pancreatic duct epithelial cells (PDEC). **Aim:** Here we addressed whether other organelles such as mitochondria and lysosomes also are controlled by the same mechanism. **Methods:** Mitochondria and lysosomes were labeled with fluorescent MitoTracker Red and LysoTracker Red, respectively. Their movements were monitored every 1.2 s with high resolution fluorescence microscopy and measured by comparing subsequent images. Cells were stimulated with UTP to activate an endogenous $P2Y_2$ G-protein coupled receptor to increase intracellular $Ca^{2+}$. **Results:** Mitochondria and lysosomes moving actively at rest stopped rapidly ($\tau \sim 2.6$ s) after $Ca^{2+}$ rise. This organelle 'freezing' was accompanied by the formation of F-actin in the whole cytoplasm as stained with phalloidin-Alexa 488. EGFP-actin binding domain 2 (ABD2) expressed in PDEC, indicated a rapid formation of cytoplasmic F-actin ($\tau \sim 7.8$ s). In addition, the freezing of the organelles was blocked by latrunculin B, an inhibitor of F-actin formation. **Conclusion:** $Ca^{2+}$-dependent formation of a fine F-actin meshwork reduces the movement of intracellular organelles physically in PDEC. The freezing mechanism controls exocytosis of secretory granules and may affect local ATP supply by controlling mitochondrial distribution in PDEC.

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## Platform BG: Microtubular Motors

**2938-Plat**

**Crystal Structure of Nucleotide-free Kinesin-1 Motor Domain Explains Coordinated Walking Mechanism**

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Kinesin is a highly processive motor that moves along microtubules in a hand-over-hand manner. To understand structural basis for the walking mechanism, atomic-detailed structural information of kinesin at various nucleotide states are essential, however the nucleotide-free crystal structure has been unavailable. Here we report the first crystal structure of kinesin-1 motor domain without bound nucleotide solved at a 2.8Å resolution. The structure fits very well to the 9Å cryo-EM density map of kinesin-microtubule complex without nucleotide (Sindelar et al. 2007), demonstrating that the structure represents the ADP-released microtubule-bound kinesin head. The nucleotide-free structure showed marked differences from the previously solved ATP- and ADP-like crystal structures. First, compared to ADP-like structure, the switch II helix extends and this extension is stabilized through the interactions with both switch I/II loops and tubulin subunits. Furthermore, switch I uncoils and extends toward the nucleotide pocket. We suggest that microtubule-binding is sensed via the extension of switch II helix, which then promotes ADP release through switch I/II loops. Another striking feature is that α6 helix that directly connects to the neck linker moved such that the beginning of the neck linker collides with the switch II helix. The neck linker itself is mobile but this steric hindrance prohibits extension of the neck linker to the forward direction. Using crystal structures docked to the EM density map, we modeled dimeric kinesin on microtubule and found that kinesin cannot adapt two-head-bound state when both heads are nucleotide-free due to the constraint posed on the neck linker, and the tethered head can bind only to the forward tubulin-binding site after the trailing head becomes ATP-like structure. These findings provide structural basis for the coordinated processive movement of two kinesin motor domains.

**2939-Plat**

**Conformational Specificity in Allosteric Signaling: High-throughput Measurement of Modular Secondary Structural Changes within Human Eg5 Kinesin**

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Communication within allosteric enzymes requires long-range interactions. The ATPase activity of human Eg5 kinesin (HsEg5) is allosterically inhibited upon noncovalent binding of monastrol or S-trityl-L-cysteine (STC) with the L5 loop and subsequent distal conformation changes. Our hypothesis is that the E116 and E118 carboxylates, flanking an isolated β-bridge in the L5 loop, are required for allosteric signaling during small chemical inhibition. Surprisingly, assessment by molecular and biochemical methods revealed that substitution of these carboxylates, irrespective of its nature, has a positional dependence on HsEg5 ATPase rates. Single-site substitution of E118 increased basal ATP hydrolysis rates of Eg5, whereas substitution of E116 resulted in lower ATPase rates. Thus, sequence variation at residues 116 and 118 of the L5 loop can drive upregulation and downregulation of ATP hydrolysis *in vitro,* respectively. We conducted high-throughput infrared measurements of the secondary structure composition of the upregulated and downregulated proteins in solution. Vibrational signatures from upregulated proteins were distinct from the net changes measured from downregulated proteins. Compared to control samples, in HsEg5 motors with E118 substitutions, amide I′ components between 1642-1620 $cm^{-1}$ decreased in normalized area, whereas proteins with alterations of E116 exhibited an increased contribution in the amide I′ area for modes between 1684 and 1646 $cm^{-1}$. Moreover, the spectral changes exhibited by E116 variants were similar to those of wildtype HsEg5 during allosteric inhibition by monastrol or STC. We conclude that allosteric inhibition by amino acid substitution or by small molecules both result in convergent steady-state changes to the secondary structure of the HsEg5 motor domain in solution, and these rapid methods will provide insight into how long-range structural changes impact motor function.

**2940-Plat**

**The Motility of Monomeric and Dimeric Variants of Eg5 studied in the Presence of the Kinesin-5-specific Inhibitor Monastrol**

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The homo-tetrameric motor-protein Eg5 from Xenopus laevis drives relative sliding of anti-parallel microtubules, most likely by the processive action of its two sets of dimeric motor domains at each end. As recently shown by Kwok et al. (NCB 2006) and Kapitein et al. (JCB 2008) , tetrameric motors move on a single microtubule in a fashion including diffusional and directional episodes, while motors moving between anti-parallel microtubules act in a highly directional and processive fashion. We have studied the processive behavior of a dimeric chimera (Eg5Kin) carrying the Eg5-motor and neck-linker and the Kinesin-1 neck and stalk. While Eg5Kin displays essentially the same motile properties as a truncated Eg5 (Eg5-513 his, Krysziak et al., JBC 2006, Valentine et al., NCB, 2006) its processivity is 40x increased to about 240 consecutive 8nm-steps on average, at a velocity of 95 nm/s. With increasing monastrol concentrations we find a dose-dependent and cooperative reduction in run length, but not in speed, indicating that two monastrol molecules are required to terminate a processive run. To further study the allosteric effect of monastrol on the motility of Eg5-motors, we generated monomeric and dimeric Eg5-constructs and compared their surface gliding-velocities in the presence of increasing concentrations monastrol.

**2941-Plat**

**Cross-Species Analysis of Kinesin-14s: Human HSET Functions in Fission Yeast to Regulate Spindle Bipolarity**

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The discovery of Kinesin-like proteins in 1990, five years after conventional Kinesin, has blossomed into the identification of hundreds of Klps that are classified into fourteen families and one orphan family. Not all Klp families are conserved between eukaryotes, however the Kinesin-14 Klps are ubiquitous and key regulators of bipolar spindle assembly and microtubule dynamics and organization. Kinesin-14 Klps associate with microtubules through ATP-dependent binding of their motor domains, but an additional ATP-independent microtubule binding site in the stalk domain is proposed to allow bundling of microtubules. Kinesin-14 members represent some of the most extensively studied Klps, however limited analysis has been done to examine conserved